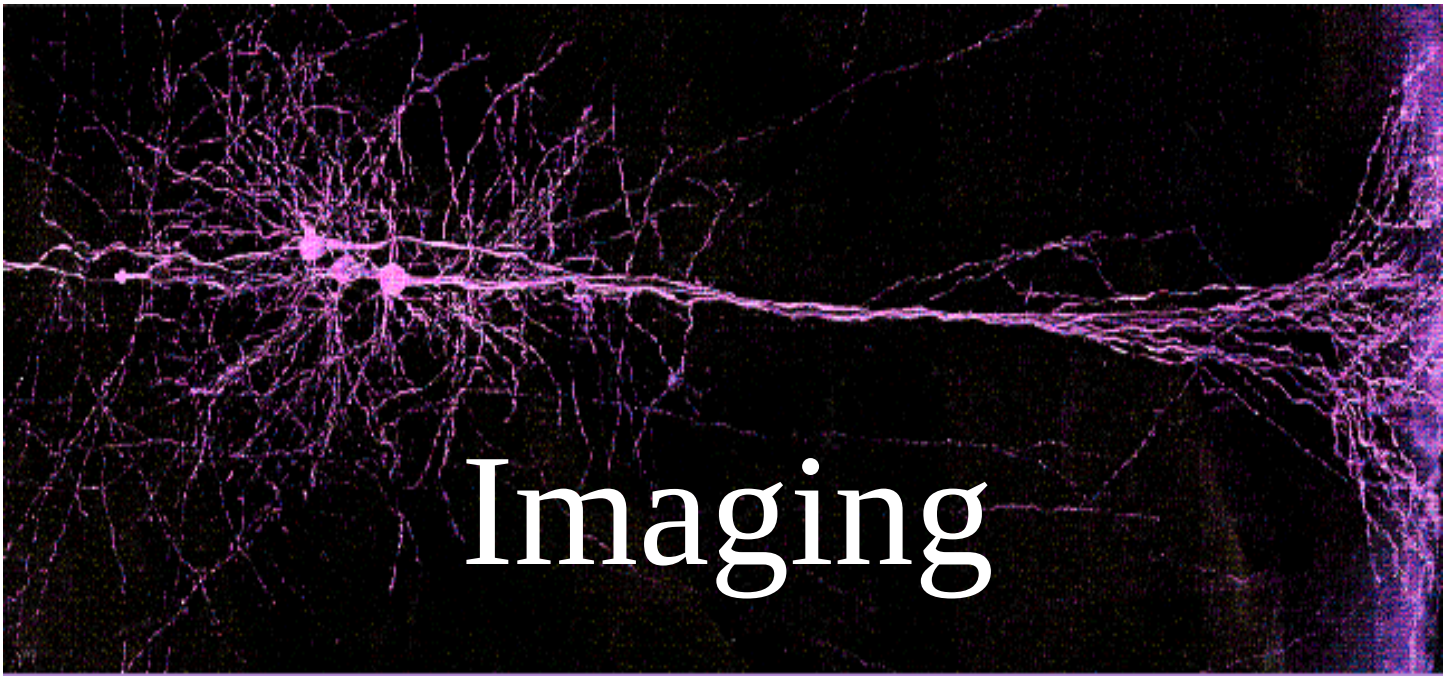


Review of Current Imaging Capabilities.

David Harper
Research Engineer, Lockheed Martin Space Operations

Directly viewing the nanoscopic structures and activities of cells is an irreplaceable part of biological discovery.

The power of imaging comes from its unique ability to provide a holistic comprehension of a highly complex picture of biology. It ties together a large number of different factors that would be difficult or impossible to piece together from individual studies. The result is an unprecedented window into the internal life of cells and each of the higher levels of biological organization. The payoff is a qualitatively higher, and easily obtained, understanding of whole complexes and systems.

The following gallery is not an catalog of imaging techniques as there are many variations. It reviews categories of *capabilities* to provide a familiarity with what current research imaging can accomplish.

Introduction.2

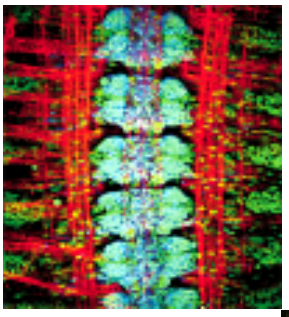
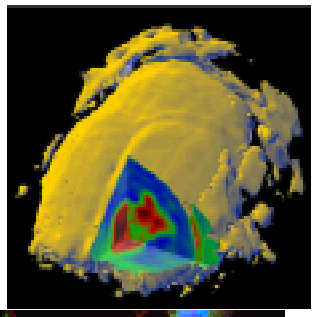
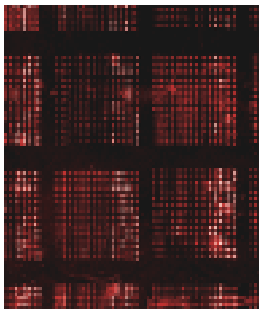
Imaging with Fluorescence and Computers

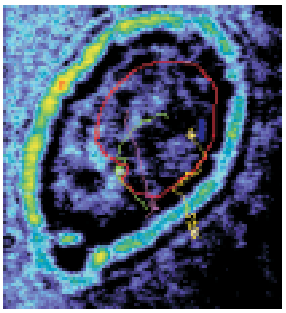
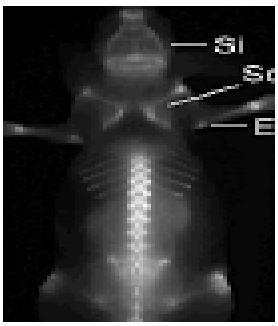
While the tools and techniques of microscopy have been steadily improving over the past decade there has also been two especially significant developments, fluorescence and computers, that have allowed great improvement in seeing particular structures and in use of the images.

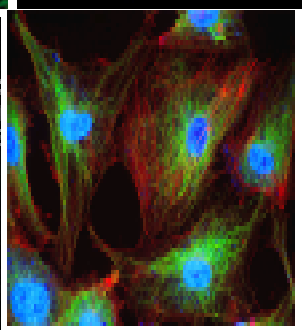
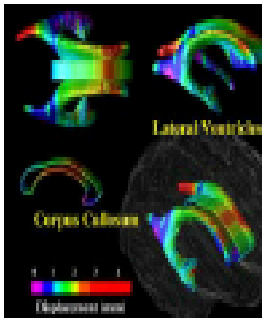
Fluorescence involves tagging, dyeing, or genetic modifications to make the bio-structures of interest glow and stand out in micro- (nano-) graphs. Fluorescence has been adapted to improve a number of different techniques: cell, molecule and virus tracking, changes in cell structures, time lapse of developing structures, activity of organs. It is even used in high throughput genomics and proteomics techniques.

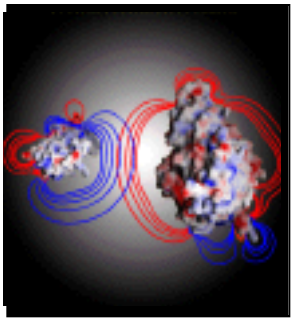
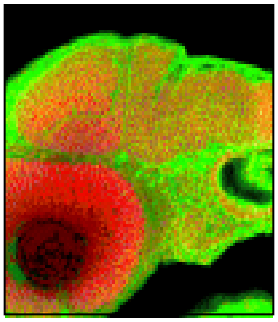
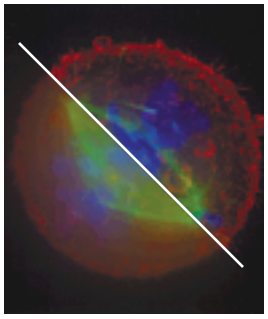
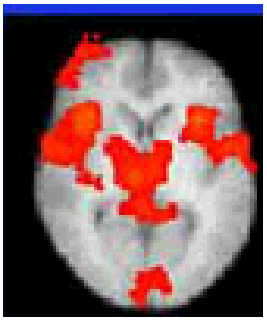
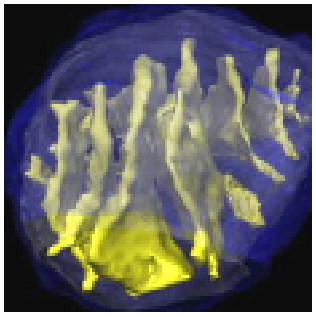
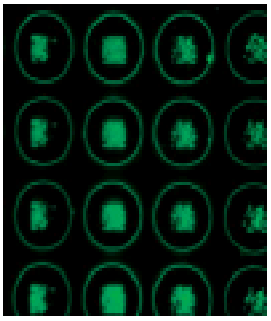
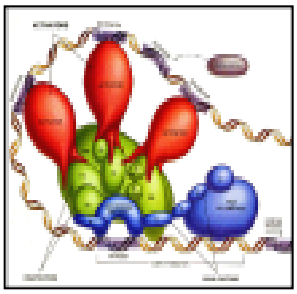
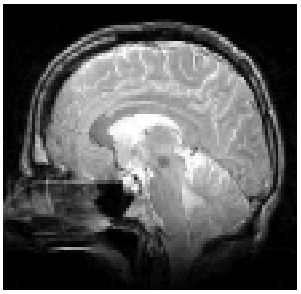
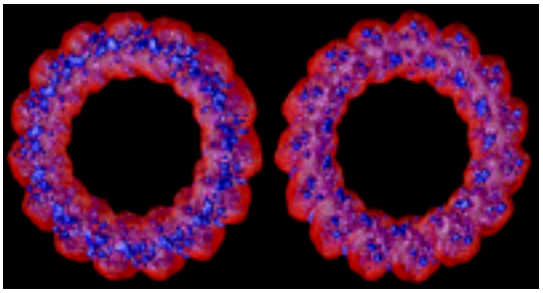
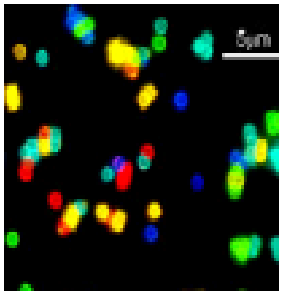
Digital representation has revolutionized the use and analysis of images by improving and/or automating a number of image aspects. acquisition; measurements; feature extraction, identification, analysis; and annotations. It allows 3D assembly of 2D slices of images, which allows virtual dissection The internet enables access to images and research from labs around the world and bioinformatics research.

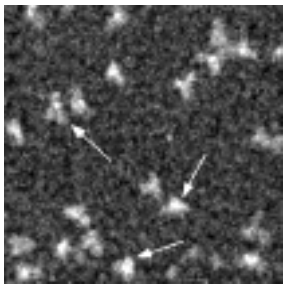
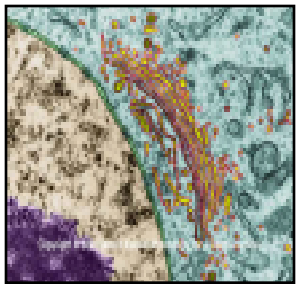
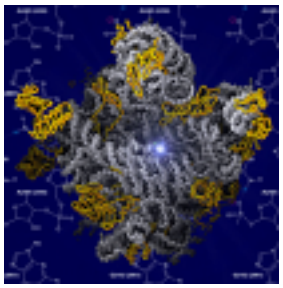
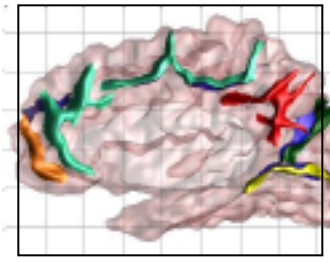
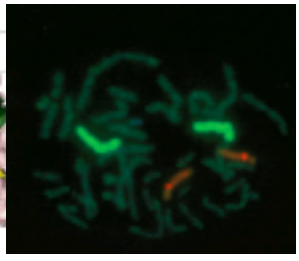
Imaging Report

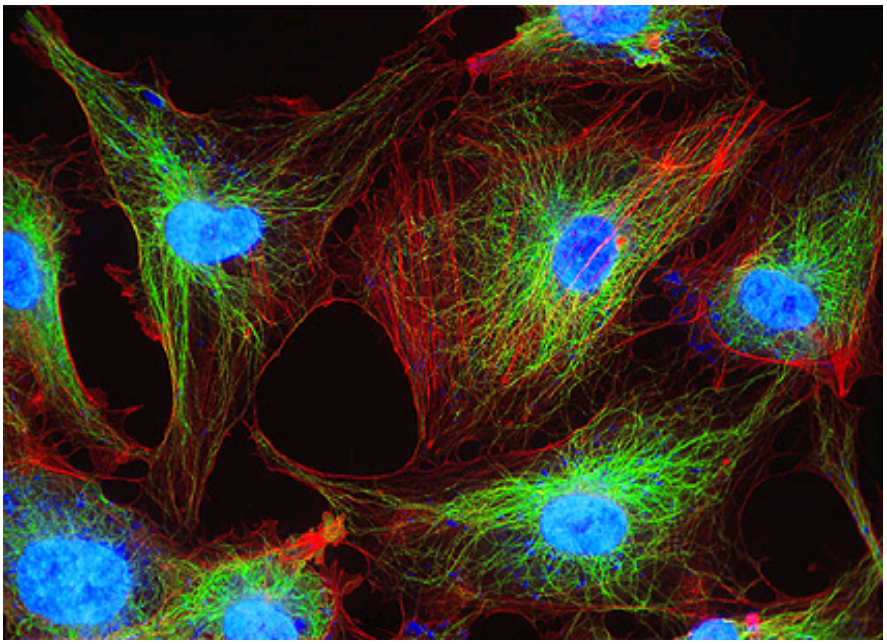
These images are discussed in the following pages. Look at this document in gallery mode to find the page

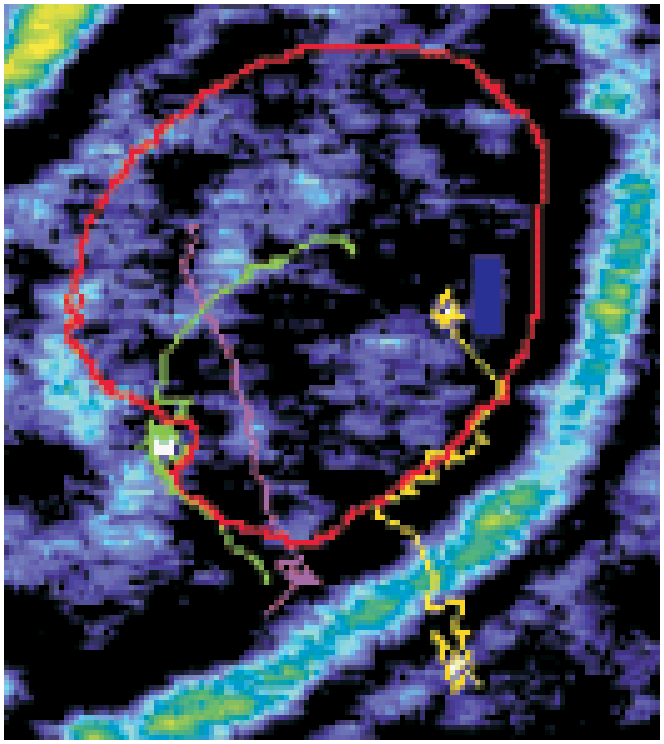
Fluorescence

Fluorescence is establishing itself as a key tool in the discovery of biology's mechanisms and structures. Researchers have developed fluorescence techniques to view the structural details of cells, to track the activity of bio-molecules, cells and viruses, to follow the development of biological systems, etc, etc.

Human endothelium cells.
http://www.probes.com/

Actin, the cell skeleton, is red.
Tubulin, the cell's highway system, is green.
Cell nucleus is blue.

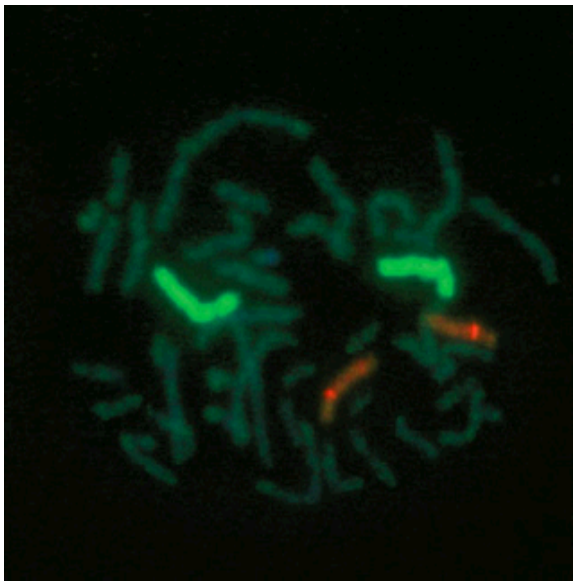


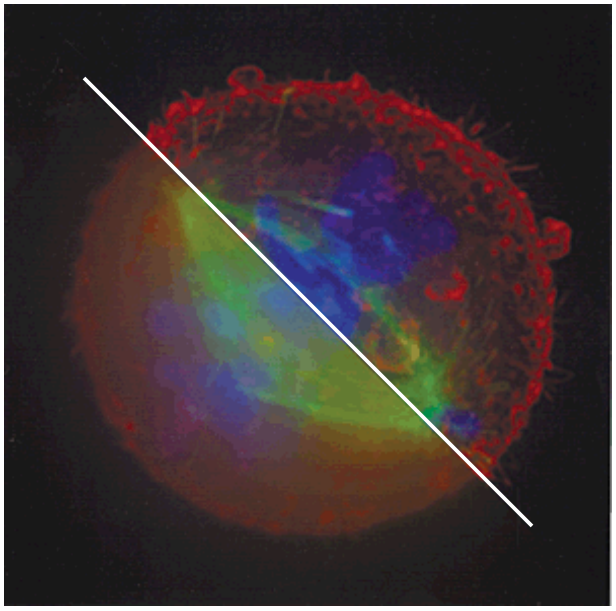


A Single fluorescent molecule is attached to 3 **viruses** and they are allowed to enter a cell. The virus is imaged over time, tracing its path. The viruses (yellow, green, and pink lines) infect one cell (outlined in yellow and aqua) and head straight to the nucleus (outlined in red)
http://www.sciencemag.org/cgi/content/full/294/5548/1803a

Fluorescence.2

Chromosome painting, using CCD-camera (Hamamatsu) .
Reference: Workshop DKFZ Heidelberg.





3D Deconvolution software compensates for light scattering that blurs images and obscures structures in fluorescence microscopy. Images have substantially less noise, better definition, and higher resolution in 3D. The software also automatically reads all recording parameters from the image data, allows a preview, focus assistance, calibration assistance, etc. http://www.zeiss.de

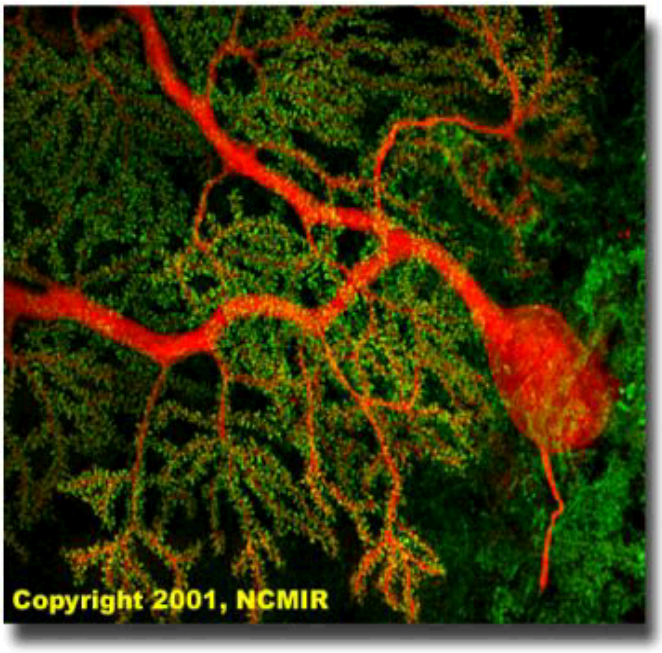
Fluorescent Labeled Trabecular Bone: the mesh of porous bone in the center of bones that contains bone marrow.

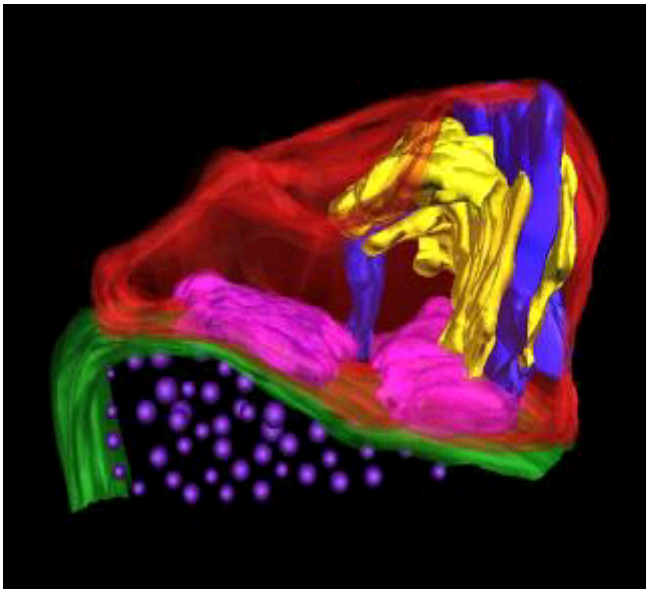
100 Microns

Fluorescence.3

Fluorescent dyeing of a cell can reveal the type and location of the different substances that make up the cytoplasm and organelles.

Projection of a series of optical sections through a Purkinje neuron revealing both the overall morphology (red) and the dendritic spines (green). The red dye fills the entire dendritic tree when injected, including shafts and spines. In contrast, the green dye selectively labels the spines.



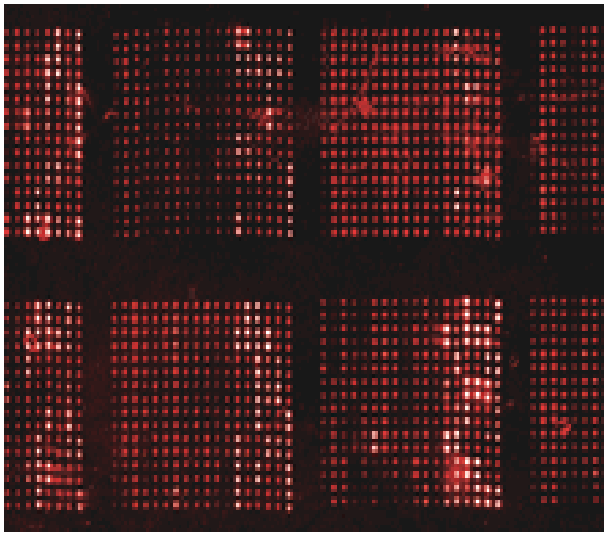


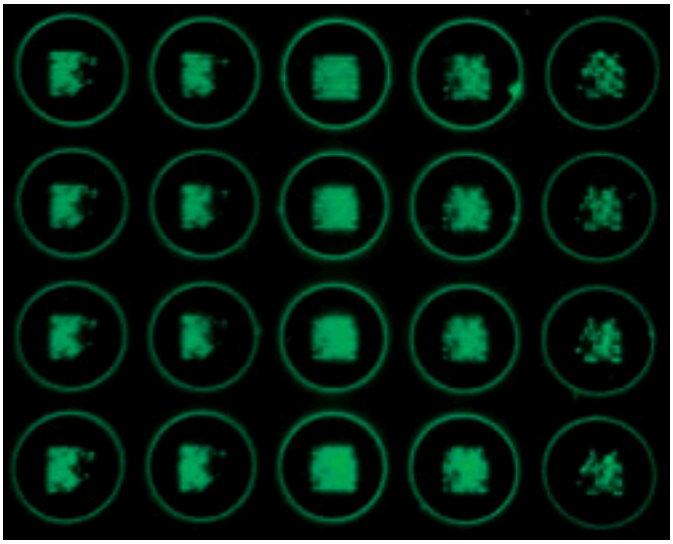
Computer rendering of Dendritic spines
http://ncmir.ucsd.edu/biology.html

Imaging and High Through-put Research

Microarrays are a key technology behind the ability to efficiently discover what and how many RNA or proteins are present in a cell. This capability allows tracking global gene expression patterns characteristic of normal and disease states, and allow time lapse monitoring of genes that are up- or down-regulated when a drug, disease or signaling factor is added.

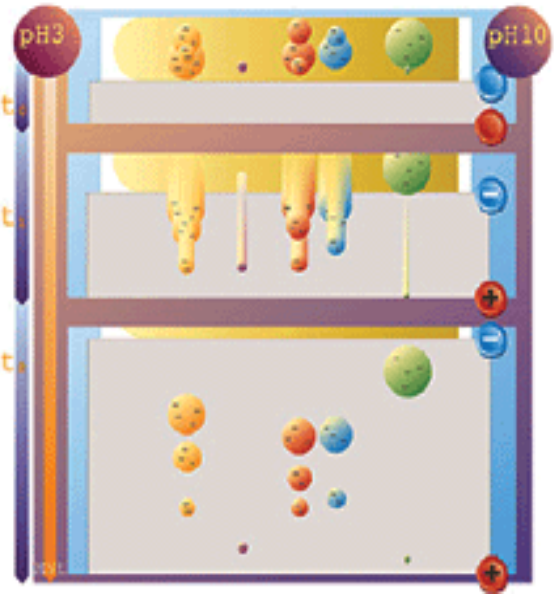
This highly parallel data taking depends on fluorescent dyes and a simple scanner to automate the data reading. The process: dye with fluorescence all molecules in a sample, put the sample on the chip, the molecules attach to the wells (only one type of RNA in each well), each molecule adds its fluorescence to the total making the well brighter and so indicating the quantity of the protein. The chip is read into a computer with a simple scanner or digital camera, where the information is processed.



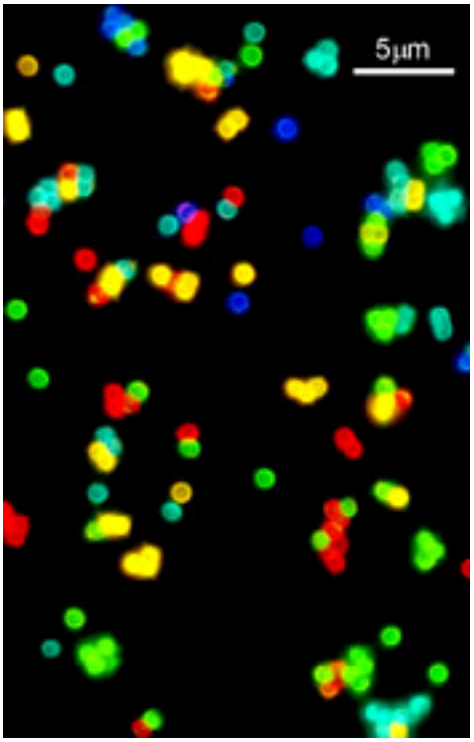


2D gels are the workhouse of protein detection. The proteins in a sample are separated by weight in one dimension and electric potential on the other. The result is a 2D pattern of protein groupings, each spot containing just one type of protein. The position of each spot indicates its protein's identity. The spots can even be removed for other studies. There is now a database of these gel images that can be used for bioinformatic comparison against all stored gels.

For comparisons (e.g., diseased vs. normal cell) two or more samples can be dyed different colors and be processed together in a single gel. The changes in protein populations are easy to pick out.



Imaging and High Through-put Research



Quantum Dots

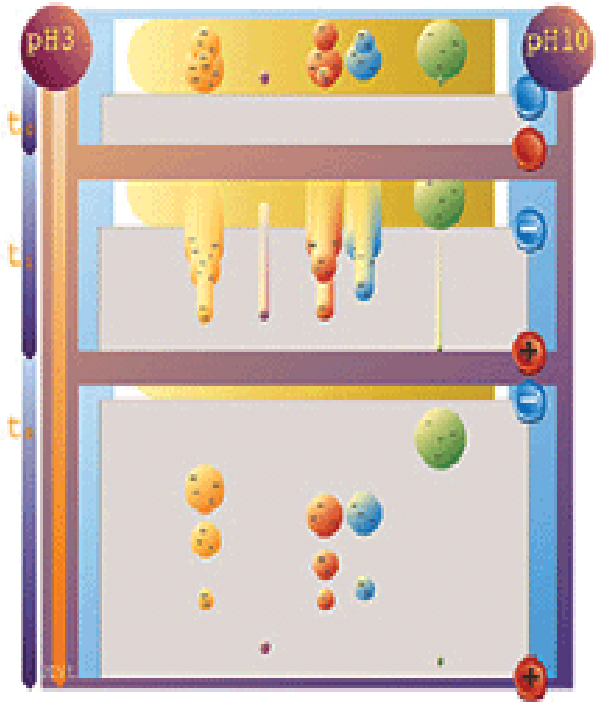
http://www.nature.com/nsu/010705/010705-4.html
Latex beads with even tinier light-emitting crystals called quantum dots, can serve as microscopic bar-codes. When attached to DNA probes or to antibodies, a bead can home in on a target molecule, tagging it with a code.

Quantum dots are tiny crystals of cadmium selenide just 200 to 10,000 atoms wide wrapped in zinc sulphide. The size of the quantum dot determines the color of the light it emits.

They are inserted into the pores of tailor-made latex micro-beads. Varying the size and number of dots in each bead, bead-dot amalgams can produce ten different light intensities and six different colors. Combinations of colors and intensities can tag up to a million unique molecules.

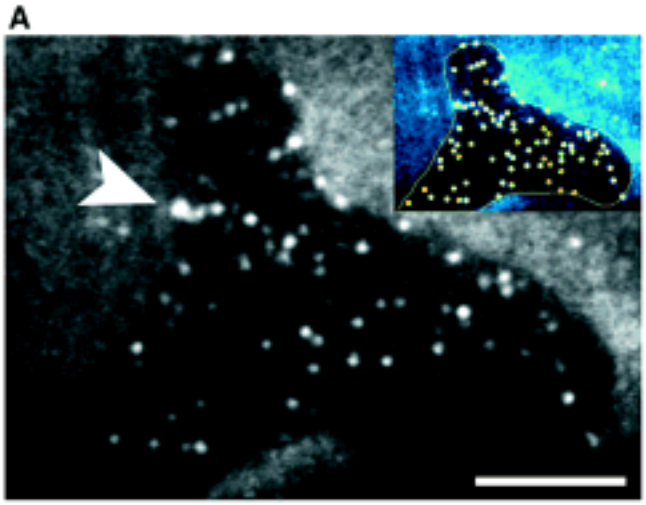
2D gels are the workhouse of protein detection. The proteins are separated by weight in one dimension and electric potential on the other. The result is a pattern of protein groupings teach containing just one type of protein. The position of each spot indicates its protein's identity. The spots can even be removed for other studies. There is now a database of these gel images that can be used for comparison against all stored gels.

The image at right shows 4 different samples on one gel. Each sample is stained with a different color. This provides the experimenter with a visual indication of the difference between different samples' protein populations



Single Molecule Movies

This is another use of fluorescent microscopy. Single molecules of one kind of protein are tagged with a fluorescent dye and then observed as they move through the cells.



Single Molecule Imaging
Single molecules of cAMP were tagged with a fluorescent dye and imaged over a few seconds. The cAMP was tracked as they interacted with docking points, or receptors, on the surface of amoebae.

The cAMP bound to its receptor, then moved within the cell membrane before dropping off at random. Among other things, the images prove that receptors move, or diffuse, within cell membranes.

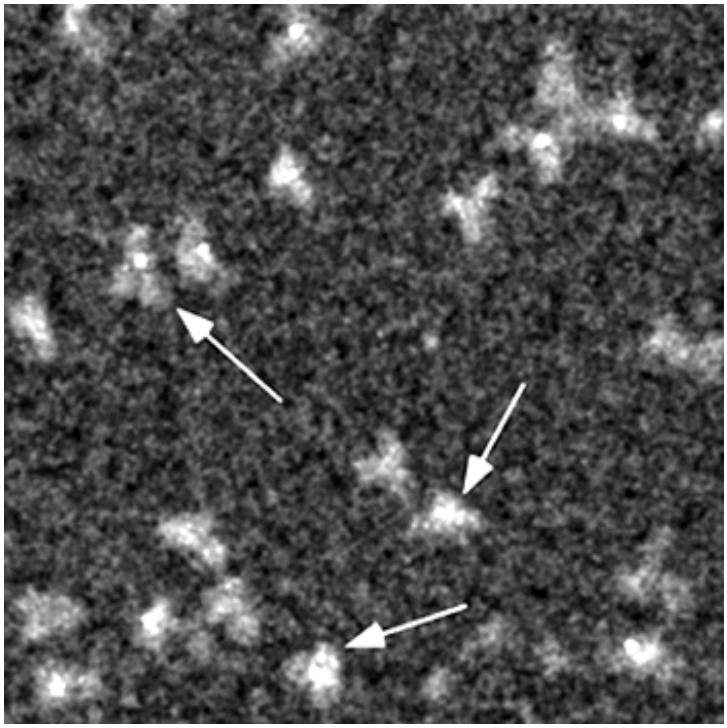
(Movie is downloadable from this URL)
http://www.hopkinsmedicine.org/press/2001/OCTOBER/011029.htm

This movie observes all the cell-membrane proteins in a cell while they journey from the cell ER to the Golgi Apparatus then to the cell membrane where they disperse the proteins like a rocket exploding against a wall and then they self-assemble in the cell wall.

Imaging Molecules

Labeling specific structures with fluorescent tags allows detailed observations in what otherwise is a very noisy field. It also can become a direct research tool as a sensor for molecules and structures of interest.

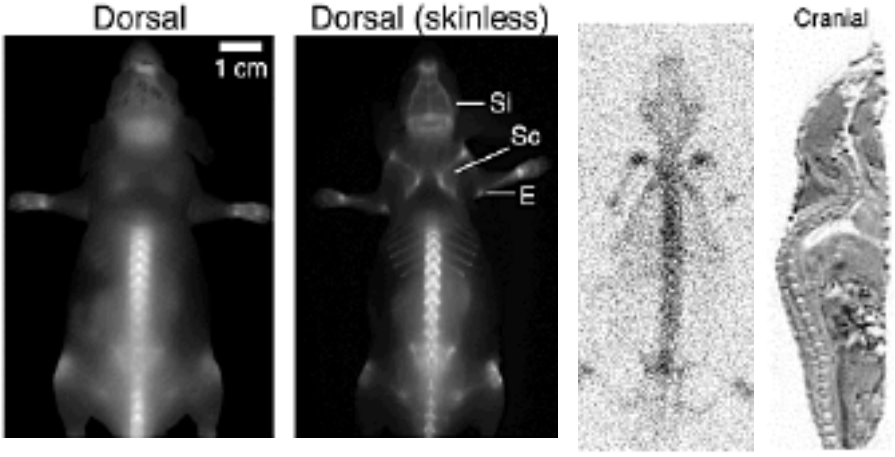
This **scanning transmission electron microscope** (STEM) image indicates that labeling with NANOGOLD monomaleimide (N-20345, arrows) occurs specifically at a hinge-thiol site on the IgG molecule. Image courtesy of Nanoprobes, Inc.



Atomic Force Microscopy (AFM) measures the height of peaks and valleys of an atomic terrain by passing a probe across the ‘’terrain’. This image shows the surface of the d. radioduran’ membrane structure.

AFM has been used to virtually place a researcher into a real time atomic environment using existing **virtual reality** technology. This allows the free movement of the AFM probe among the atoms and even allows the researcher to push the atoms around.

Live tissue



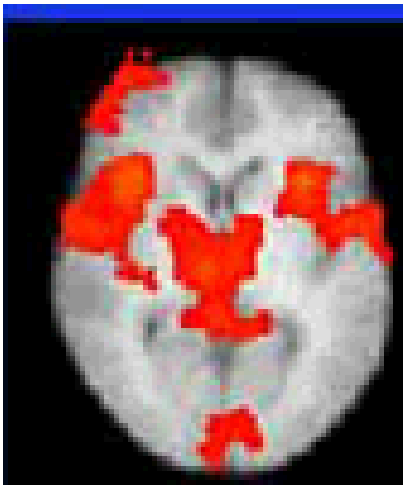
A. **In vivo near-infrared fluorescence imaging of osteoblastic activity**. (When the bone forming cells are releasing its main bone forming molecule, it glows.)
B. Comparison image using radiological technique.
C. MRI for mapping skin, muscle etc, position and thickness. To factor

Micro-endoscope: Real-time fluorescent confocal microscopic imaging of living tissues.

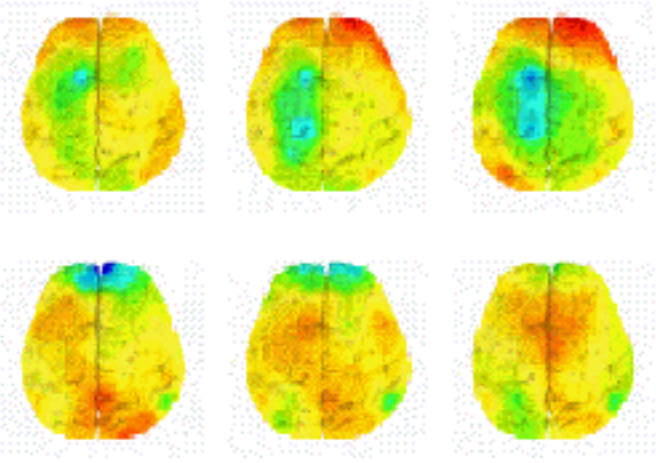
A camera and cell collector that is a few human hairs in width is in testing phase. Developed for breast cancer detection by entering the nipple to examine ducts - the typical location of cancer development. May replace biopsy as the primary method of examining mammogram irregularities. It has promise as a generally applied, minimally invasive procedure for examination and sample collection.
http://www.optics.arizona.edu/gmitro/Medical/medical.htm

A Miniature Head-Mounted Two-Photon Microscope: High-Resolution Brain Imaging in Freely Moving Animals
TRL 4
http://www.neuron.org/cgi/content/abstract/31/6/903/?highlight=denk
Surgically mounted microscope allows direct viewing of neurotransmitter concentration changes in an intact synapse in a otherwise normal rat. By using the microscope and a fluorescent dye to measure calcium concentrations in receiving cells, biochemical changes can be viewed that dictate whether or not the cell will fire. It can penetrate up to 200 micrometers into the cortex (a synapse rich environment), it has a resolution of 20 micrometers. Bell Labs

Live Tissue

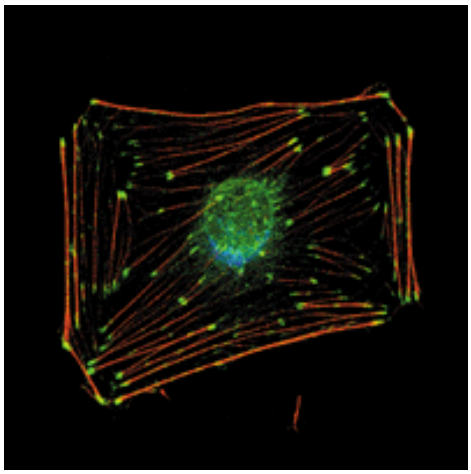


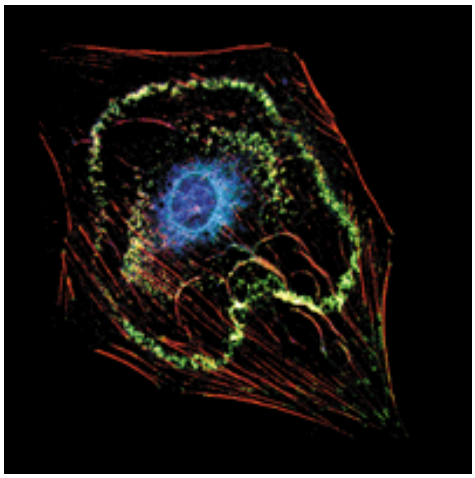
Functional Magnetic Resonance Imaging can monitor the activity of the brain by measuring the amount of waste products being produced.



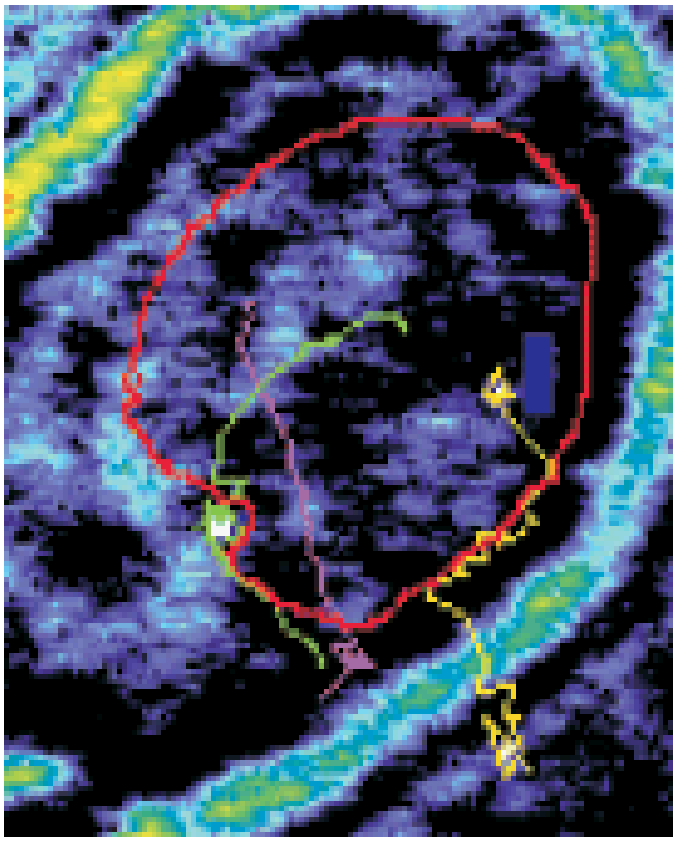
Electroencephalogram (EEG) showing the difference in brain activity patterns between a placebo (bottom row) and a anti-depressant (top row). The blue shows higher levels of activity and red indicates low activity.

Time Lapse Imaging.2





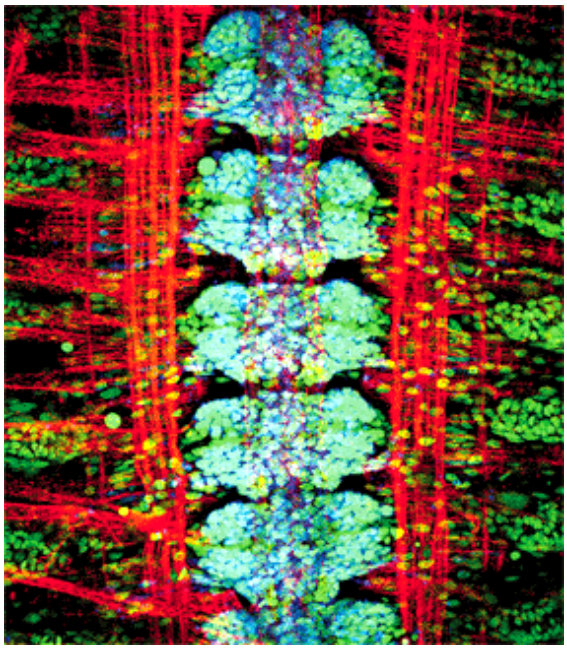
FLOW OF MESSAGE in a skin cell was made visible by coloring two components of a signaling pathway: a scaffolding protein (green) and one of two enzymes tethered to that scaffold (blue). Actin, a structured element in cells, is red. The left cell is quiet. Soon after an external messenger activated a signaling pathway in the right cell, the scaffolding protein moved its bound enzymes to their targets deeper in the cell. That movement is revealed by the yellow hue, which derives from the overlap of the colored signaling components with actin (to which the enzymes' targets were bound). The blue mass at the center reflects extra copies of the colored enzyme



Tracking viruses

3 viruses were tagged with a single yellow, green or purple fluorescent molecule and allowed to invade a cell and then a nucleus (outlined in red). The virus' are seen tapping and probing the membranes until they penetrate.

Time Lapse Fluorescent Imaging



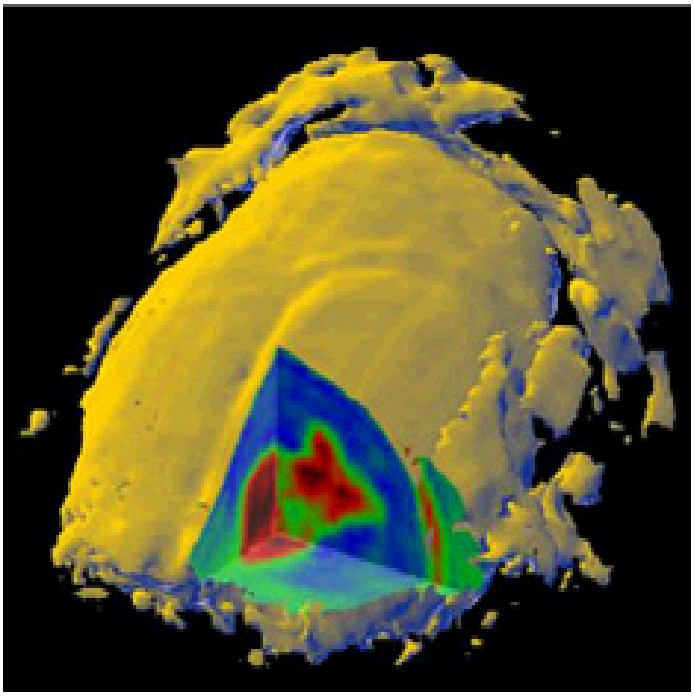
Tracing development

Fluoro-emerald (blue) was injected into a neuroectodermal cell of a leech embryo. The teloblast eventually differentiated into five segmentally iterated ganglia. Muscle fibers are labeled with red. Nuclei are green. Image reprinted from the cover of Development 127 (4) (2000), The Company of Biologists Ltd.

http://www.probes.com/servlets/photo?fileid=g001192

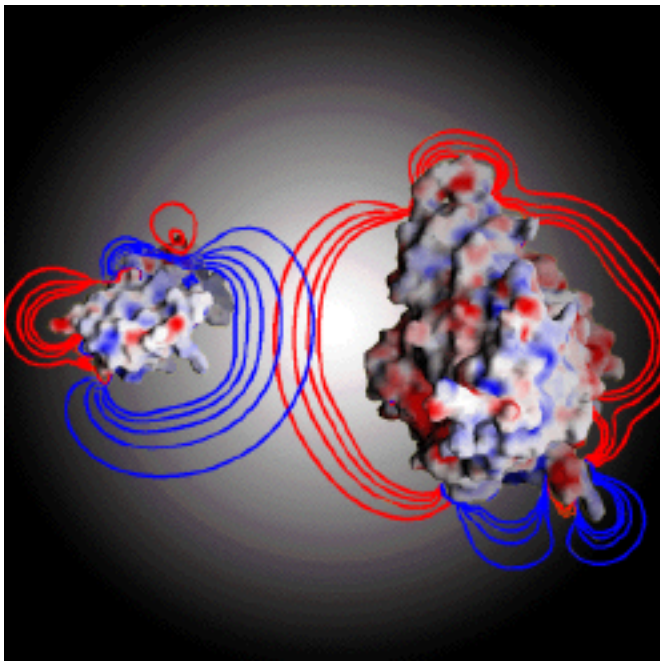
Tracking stem cell movement

Neural Stem cells absorbed iron particles and were injected into a rat brain. The iron particles showed up black on MRI images, which made the stem cells easy to track as they migrated. A computer combined the images into a 3D object that can be sliced and manipulated.

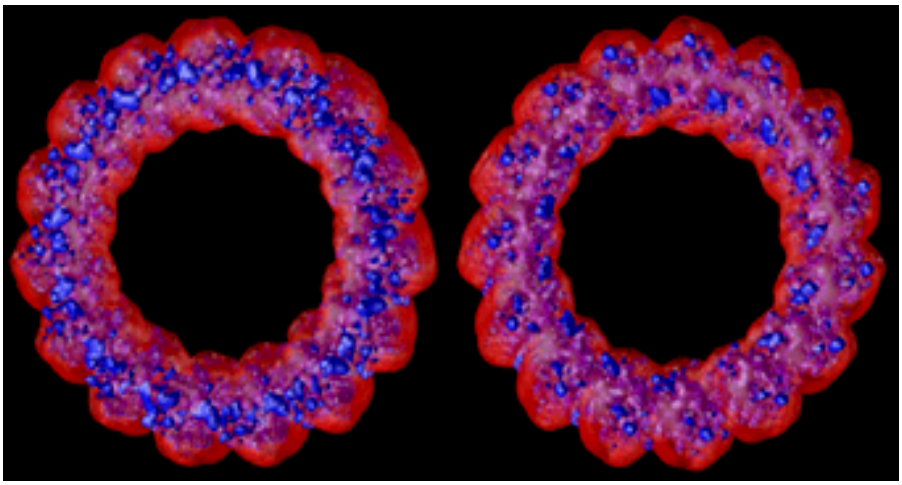


Viewing Electrostatics

Electrostatic forces speed the binding of some biomolecules by a factor of more than 100.
http://mccammon.ucsd.edu/gallery/



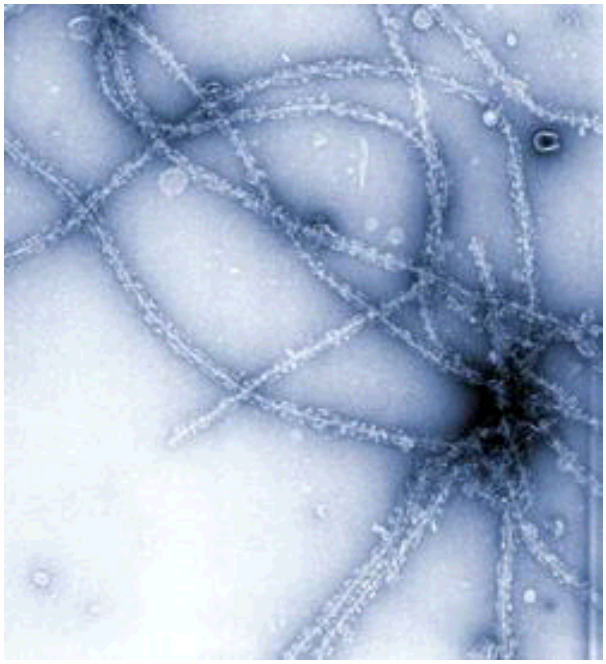
AChE and Fasciculin 2 bind with electrostatically-steered, diffusion-controlled kinetics. Protein association is of great importance in cell biology, and Brownian dynamics simulations are proving useful in the study of such associations.
http://mccammon.ucsd.edu/gallery/005.html



The electrostatic landscape can be mapped to discover the role of electric potential in assembling structures, These are the 2 ends of a microtubule, the cell's highway for moving proteins etc., They assemble adding one protein at a time in spiraling assembly.

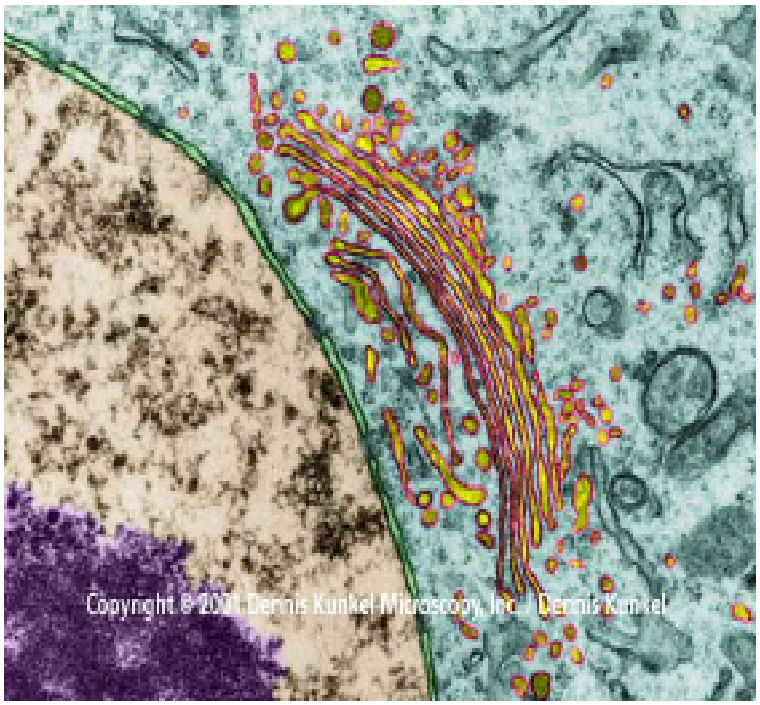
Electron Microscopy

Transmission electron micrograph of Sup35 protein that has formed amyloid fibers from its prion structure



Transmission Electron Microscopy

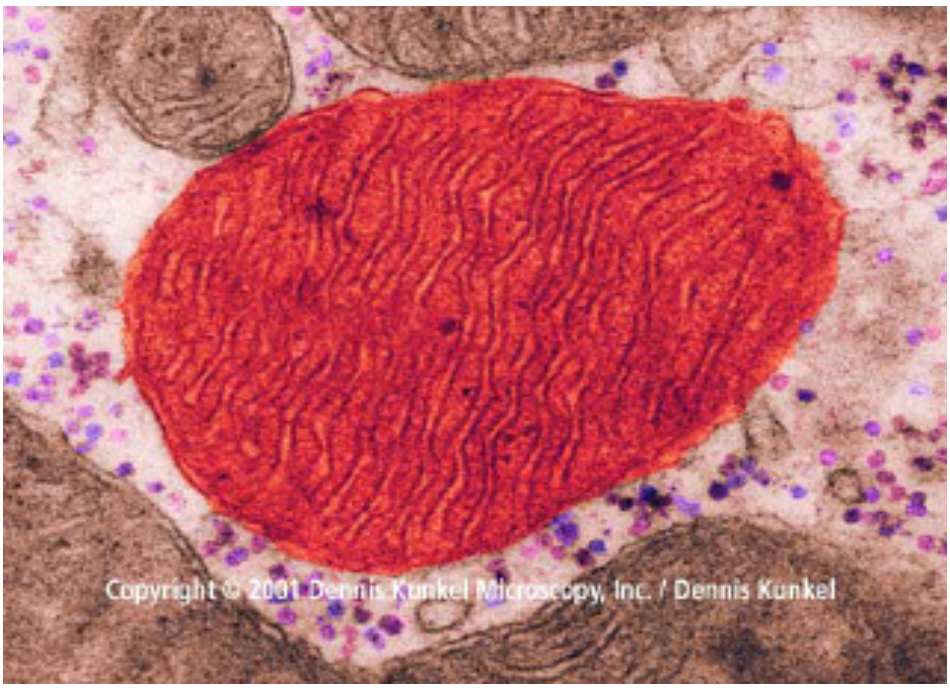
Electrons are used in the place of light because their much higher frequency can ’see’ much finer detail. These images show how detailed can TEMs can get.



Golgi apparatus are stacks of cisternae (thin membrane folds), and vesicles (free floating membrane globs) near the nucleus of a nerve cell. The nuclear membrane (boundry between green and white areas has pores in it.

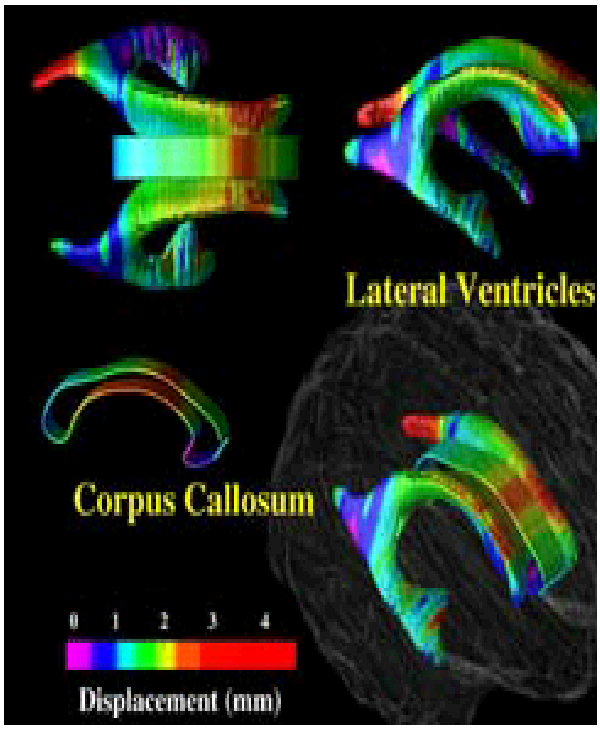
TEM Magnification:-- x9,500-- (Based on an image size of 1 inch in the narrow dimension)

Mitochondrion from an heart muscle cell showing numerous cristae. TEM Magnification:-- x25,460--(Based on an image size of 1 inch in the narrow dimension)



Imaging + Computers

Automated feature extraction, identification, analysis tools, 3d models, virtual dissection, time-lapse reconstructions, comparisons, worldwide access to image databanks, statistical analysis, virtual reality 'insertion' into any environment that can be imaged, including live.

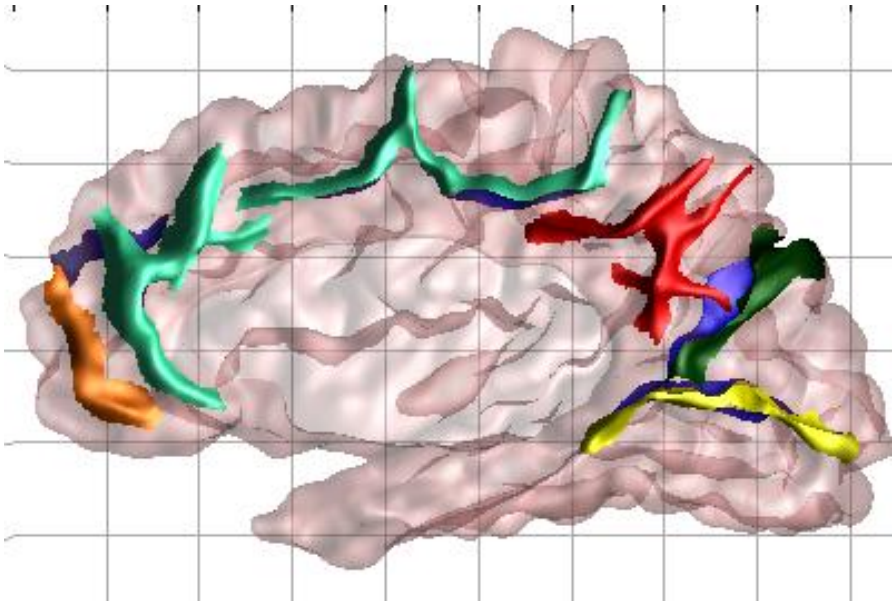


MRI's were taken of a normal and a schizophrenic brain. Using a computer, the corpus callosum was cut out of every MRI slice and those sections were assembled into 3D images.

This comparison image was created by superimposing the 2 MRI brain scans and measuring the difference at each point. The colors reflect varying degrees of difference, 0 (purple) to 4.5 (red) mm between the 2 MRI images."

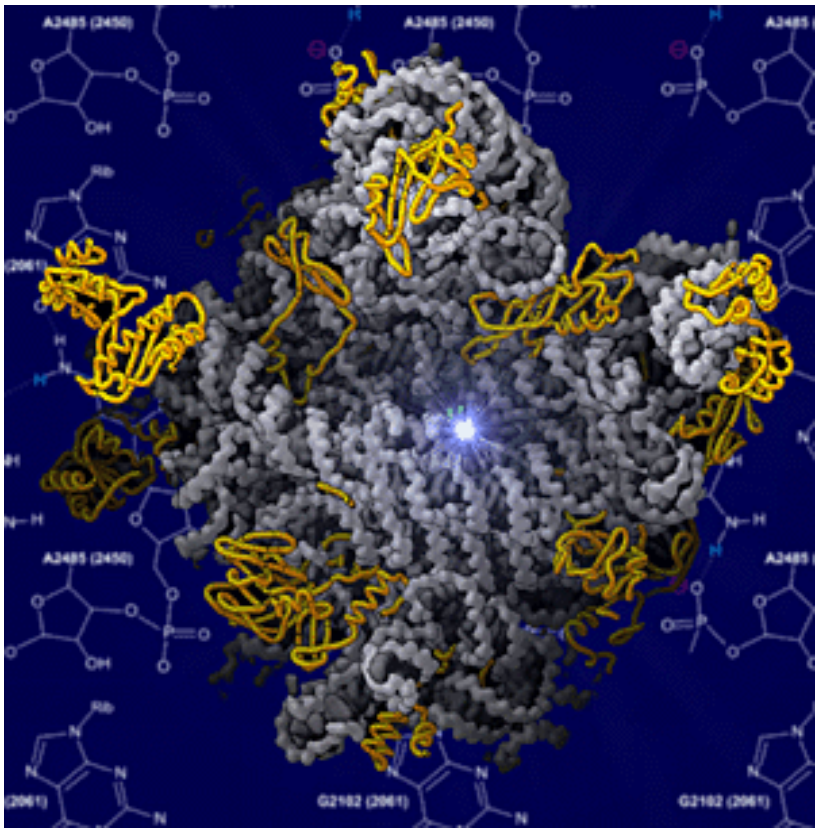
The digitized information can be further manipulated with virtual dissection.

Feature extraction
This image is part of research into developing fully automatic methods to segment and chart the cerebral cortex from magnetic resonance images, and to map and label the major geometric structures on the cortex using a detailed analysis of cortical geometry.



http://iacl.ece.jhu.edu/projects/images/bmap.jpg
http://iacl.ece.jhu.edu/projects/pdf/#pubs

Imaging + Computers



Protein Imaging
Computer Assembled Protein Structures From Crystallography data.

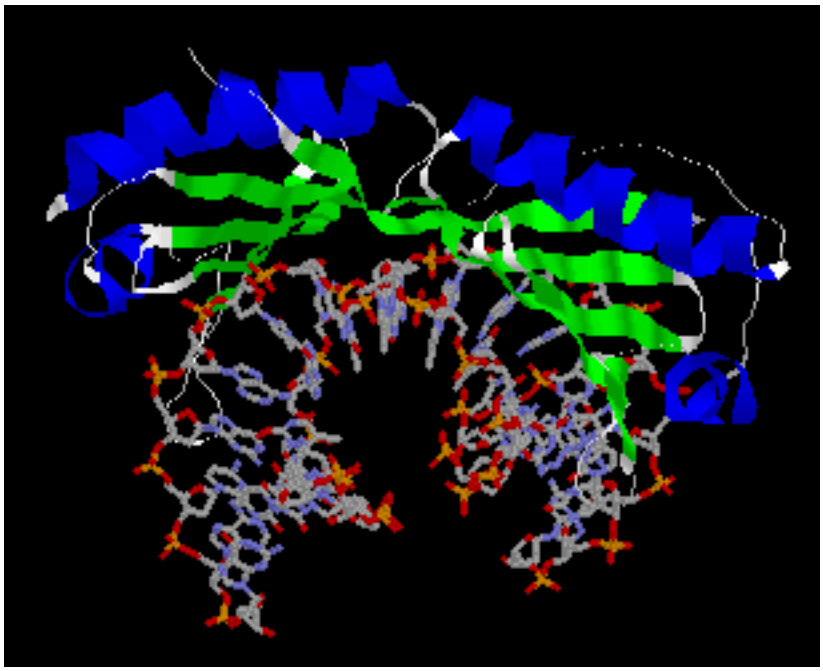
Ribosome

This image was generated by computer after the details of the ribosome's shape were determined after a long effort. The bright spot in the middle is where the the ribosome pulls the Messenger RNA past and strings together amino acids. These strings of amino acids next fold up to form proteins.
Using a technique called **3D cryo-electron microscopy**, researchers have detected a ratcheting rotation deep inside the cell's tiny protein-making "factory" at a key point in the protein construction process.
Joachim Frank, Rajendra Kumar Agrawal
July 20, 2000, Nature.

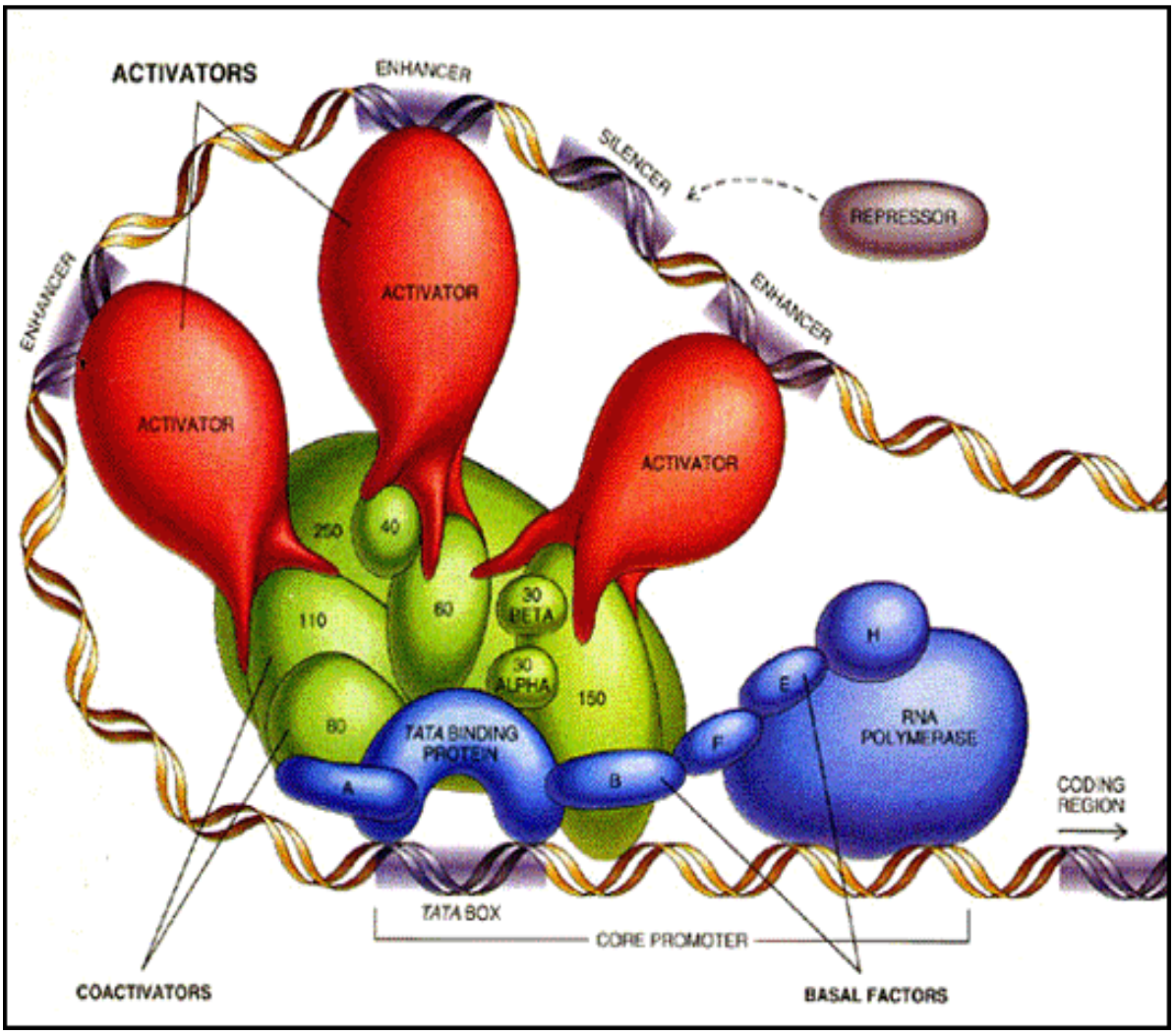
Computer generated image of a TATA Box Binding protein attaching to DNA in preparation of expressing the gene.

This type of computer modeling is essential to viewing the crystallography-generated structure of proteins and then virtually viewing its activity. This image came from an a brief movie showing how the protein squeezes the DNA into a tighter curve.

http://www.biosci.uq.edu.au/Html/Images/Galleria/kolle/assignment.html



Assembling the Bigger Picture



This shows the assembly of proteins that selects genes and marks the beginning of the coding region. .

Movies of Cells

Double click on the image to run the movies.

Cell movement is one of the original questions in biology. This movie shows the activity of actin in a moving cell.

3D mitochondria

Digitized data allows many opportunities to manipulate the images. This mitochondria can be rotated to view any angle.

Double click to rotate mitochronria

Movies of Cells

This movie shows the movement of cell membrane proteins from the ER to the Golgi and then being trucked to the cell wall where the protein package disperses (between the pointers at the image bottom) and self assembles into wall.

Movie of plant cell division.

Double click on the image to see the movie.